Heavy metal removal from polluted waters using *Schoenoplectus californicus* and *Phragmites australis*

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Abstract

Acid mine drainage (AMD) is one of the most significant environmental challenges facing the mining industry worldwide. Furthermore, as in many countries, the relationship between the mining industry, land, water and communities is very close. In Peru this relationship goes beyond economic factors. On several occasions the negative effects of AMD can reduce the quality of water resources and affect communities. For this reason, many methods have been developed for AMD treatment, being wetlands a good option for heavy metal removal. In wetlands, the use of green plants and their associated microbiota seems to be a good technique to remove, contain, or render harmless environmental contaminants.

In this paper, we present the efficiency of *Schoenoplectus californicus* and *Phragmites australis* in a laboratory test for removal heavy metals from an aqueous solution. In fact, both plants, as Peruvian native Andean plants, were tested using artificial solutions containing heavy metals such as copper, iron, lead and zinc. From binary solutions of copper-iron, zinc-iron and lead-iron the *Schoenoplectus californicus* remove 82%, 75% and 88% of copper, zinc and iron respectively; while the recovery of copper and lead in quaternary solution of copper-zinc-lead-iron was 90 and 92% respectively. On the other hand, in the case of *Phragmites australis* recoveries are slightly lower. From binary solutions the removal was 68% of copper, 53% of zinc and 20% of lead; while the recovery in quaternary solutions was 97 and 95% for copper and zinc, respectively. According to the results obtained, it is concluded that the use of *Schoenoplectus californicus* and *Phragmites australis* have a potential to be used in wetlands especially in high Andean mining areas in Peru; and thus, avoid possible contamination of natural water bodies in that area.

Introduction

The acid mine drainage (AMD) and the possible contamination of nearby water sources and soils is one of the more serious environmental problems in the mining industry. For this reason, many remedial treatments of AMD have been developed in recent years (Rodríguez-Galan, 2019). AMD remediation techniques can be classified as active and passive treatments, being the active methods the most widely used (Obreque-Contreras, 2015). Passive treatments, being relatively inexpensive and environmentally friendly, have recently become a viable implementation alternative. There are several types of passive treatment systems (Costello, 2003; Sheoran, 2006; Skousen, 2017; Ziemkiewicz, 2003) that are summarized in Table 1.

Table 1. Passive treatment systems.

Passive Treatment Methods	
Aerobic wetlands (AeW)	Open Limestone Channels (OLC)
Anaerobic wetlands (AnW)	Permeable Reactive Barriers (PRB)
Anoxic limestone drains (ALD)	Pyrolusite® limestone beds
Reverse alkalinity Producing Systems (RAPS)	Slag leach beds (SLB)
Limestone Diversion Wells (LDW)	Successive alkalinity producing systems (SAPS)
Limestone Leach Beds (LSB)	Sulfate reducing bioreactors (SRB)
Microbial Reactor Systems (MRS)	Vertical flow wetlands (VFW)

In wetlands, the use of green plants and their associated microbiota seems to be a good technique to eliminate or reduce the concentration of environmental pollutants (Hallberg & Johnson, 2005; Klein, 2014; Mojiri, 2017). In addition, there are several factors that influence the effectiveness of plants. In fact, ideal plants must be hardy; able to tolerate low nutrient levels, be resistant to weather shifts, and uptake more contaminants than normal plants. Different plants for heavy metals removal have been evaluated in pilot and constructed wetlands (Table 2).

Table 2. Plants used in passive treatment systems.

Plant	Reference
Cyperus esculentus	Folsom, 1981
Eichhornia crassipes	Mishra, 2008
Phragmites australis	Bonanno, 2010; Lee, 2007; Southichak, 2006
Pista stratitoes	Miretzky, 2004
Schoenoplectus californicus	De Lange, 1998; Vymazal, 2013
Scirpus lacustris	Vymazal, 2011
Typha domingensis	Dunbabin, 1992; Vymazal, 2011
Vetiveria zizanioides	Borralho 2020

Being *Phragmites australis* and *Schoenoplectus californicus* being the most used plants due to their wide tolerance to changes in pH (4 - 10), salinity (20 – 45 mg Cl/l), temperature (10 - 32ºC) (Neubauer, 2012; Reddy, 1990; Reddy, 1997; Stein, 2005).

Schoenoplectus californicus growths in coastal and riparian regions from southern North America (Manson, 1957) to Chile and Argentina (Wagner, 1990). This plant is also found in some Pacific islands such as the Cook Islands, the East Island, New Zealand and Hawaii (Hidalgo-Cordero). It also frequently founded in high Andean areas of South America (Blanco, 2019). In fact, this plant is found at elevations from sea level to 3800 m.a.s.l.. Growing in extreme habitats, *Schoenoplectus californicus* has developed the ability to withstand fluctuations in temperature and volumes of water, high levels of ultraviolet-visible (UV-Vis) radiation, high salt content (areas near the beach) and potential polluting elements such as arsenic of natural origin. (Montoya, 2009). *Schoenoplectus californicus* is a perennial herbaceous plant. Its stem can measure between one and four meters and occasionally reaching 6 m depending on the variety. The habitat of *Schoenoplectus californicus* are marshes, the banks of rivers and lakes, as well as areas prone to seasonal flooding. Its roots can extend to great depths of water that vary from 2.5 to 3 m; although this plant can grow and develop without problems with water depths between 30 cm and 70 cm (Hidalgo-Cordero, 2017; Neubauer, 2012).

It is believed that *Phragmites australis* was a plant native to Europe and the Canary Islands, and from there it was introduced to other parts of the world. It is currently one of the largest plants in the world. Specimens can be found from greater than 70°N to the equatorial regions and south to Tasmania (Australia) and the cone of South America (Weber, 2003). *Phragmites australis* is a plant with great adaptability to fresh water, brackish water (up to 10,000 ppm. total dissolved salts) (Sainty & Jacobs, 1988) and alkaline wetlands in the temperate zones world-wide (Roman, 1984). It also grows in natural and artificial wetlands, ditches, marshes, swamps, bogs, and prairie potholes as well as in artificial canal systems in agricultural areas (Roman, 1984). Growth of *Phragmites australis* has been reported in various climatic zones ranging from cold zones between 70°N and 43°S (Dahl 1934; Haslam 1972; Isacch, 2006; Parker, 2017) to tropical zones as Equator (Haslam 1972) and arid zones as Australia (Davies, 2010; Pfadenhauer, 2014). *Phragmites australis* is a grass species that can grow up to four meters in height (Hanganu, 1999). It reaches high densities and form monospecific stands (Hudon, 2005). The phragmite family have an estimated average lifespan of 4.5 years, but can live up to 6 years. (Haslam, 1972). It has been shown that it is possible to adapt *Phragmites australis* to altitudes between 400 to 2360 m.a.s.l. (Jiao, 2020; Klimeš, 1999; Klimeš, 2000).

Due to their great versatility and adaptation to different climates and temperatures, this study has used *Phragmites australis* and *Schoenoplectus californicus* for the design of wetlands. The aim of this research is focused on the evaluation of heavy metal removal by using *Phragmites Australis* and *Schoenoplectus Californicus* in laboratory wetlands under controlled conditions.

Materials and methods

Collection of plants

Phragmites australis and *Schoenoplectus californicus* were collected in the southern area of Lima, Peru. The collected plants were washed with tap water and deionized water, in order to remove dust.

Wetlands systems

The design and construction of the cells of polycarbonate with a width, height, and length ratio of 1:2:4 respectively were fabricated according to EPA recommendations (EPA, 2015). Four specimens of each plant were placed on the cell considering a distance of 20 cm of separation between them. Then, 15 liters of an artificial effluent containing copper sulfate (0.063 mM), zinc sulfate (0.062 mM), iron sulfate (0.062 mM) and lead sulfate (0.033 mM) was added at 3 ml/min rate. This flow was simulated in order to generate an adaptation of the plants to the environment. The facility of the wetlands is presented in Figure 1.

Figure 1: Wetlands facility details.

The experiment was carried out at 16°C to 18°C, 450 m.a.s.l., and 65% of humidity approximately. These are conditions that are similar to those of some mining operations that take place in Peru. In fact, mining in Peru takes place mainly in the high Andean zone (above 3,800 m.a.s.l.). However, some important operations are carried out on the coast with latitudes that range between 50 and 1000 m.a.s.l.. Among them we can mention: María Teresa (25 - 200 m.a.s.l.), Condestable (100 and 450 m.a.s.l.), Veta Dorada (115 m.a.s.l.), Los Incas (585 m.a.s.l.), San Juan (800 m.a.s.l.), Doble D (1200 m.a.s.l.) and Cerro Lindo (1,820 m.a.s.l.). Likewise, San Vicente (1,400-2,000 m.a.s.l.) and Palmapeta (1,500 m.a.s.l.) are located in the edge of the jungle, where humidity (90%) can be very similar to that of the city of Lima.

Water and plants sampling

Water samples from the eight constructed wetlands cells were collected in 100 ml plastic bottles during 7 days. 80 ml of each sample was filtered using 0.45 µm cellulose acetate filters (Sadler, 1992). The other 20 ml was kept as an unfiltered sample and it was used to measure the turbidity. The concentrations of ammonia and nitrate were determined by colorimetric method. The remain concentrations of copper, zinc and lead in water were determined by flame Atomic Absorption Spectroscopy (AAS) (O’Halloran, 1997).

On the other hand, the plants were carefully removed from the cells, rinsed with water and divided into aerial parts and roots, and placed in paper containers. Then they were taken to an oven to be dried at 60°C to eliminate humidity. Later, by using a grinder the samples were pulverized. Before determining the heavy metal concentration by AAS a wet digestion process was performed. One gramme of dried sample was weighted into a porcelain crucible and then calcinated at 500 °C for 12 hours in a muffle (Akinyele, 2015; Hoening, 2005; Onianwa, 2001). In order to dissolve the organic material, the ash obtained was placed in a glass beaker with 15 ml of HNO_3 (1.0 M). The beaker was heated for 30 minutes in a hot plate where the temperature was increasing to 100 °C. The residue was filtered into a 25 ml volumetric flask using a filter paper (Whatman, No. 42); the volume was completed with HNO_3 (1.0 M).

Metal removal

AAS was used to determine the concentration of heavy metal in water and in plants. To stablish the accuracy of the measurement of metal concentration with this method, blanks and standards were prepared based on the standard procedures (Ipeaiyeda, 2017; Padmavathiamma, 2007; Radulescu, 2014; Soylak, 2004).

During the analysis, a blank solution with 2 ml of the 0.50% aqueous Nitric Acid (HNO_3) was prepared. Furthermore, with the dilution of 1000 ppm of the stock with 0.50% aqueous HNO_3, three standards solutions (100 mL) for each metal were prepared (see Table 3). The blank, standard (from lowest to highest concentration) and sample solutions were placed in that order on the autosampler. At the end, all absorbance results were registered.

Table 3 Instrumental conditions of the metal analysis by AAS.

Parameters	Zn	Fe	Pb	Cu
Wavelength (nm)	213.9	248.3	283.3	324.8
Calibration range (mg/l)	0.2-1	1-5	2-10	1-5
Lamp current (mA)	9	15	10	8
Slit (nm)	1	0.2	1	0.5
Flame composition	air/acetylene			
Oxidant pressure (bar)	0.758			

Results and Discussion

Metal removal

The variation of metal concentration in the different samples have been analyzed. The results obtained from the initial day and from the eighth day are summarized in Table 4 and Table 5 for Phragmites australis and *Schoenoplectus californicus* respectively. The efficiency of absorption of heavy metals by the plant is expressed by:

$$Efficiency = \left(\frac{C_f - C_0}{C_0}\right) x\ 100$$

Where, Cf and Co are the final and initial concentration of the metals in the solution. The translocation factor (TF) is defined as the concentration of heavy metals in the shoot tissue divided by the concentration of heavy metals in the root tissue (Ipeaiyeda, 2017). Taking into account that if TF>1, the plant is a hyperaccumulator; if TF <1, it is a phytostabilizer.

- ***Phragmites australis***

The variation of the metal concentrations in binary and quaternary solutions are indicated in Fig. 2.a. The removal of copper is greater in the quaternary solution than in the binary one. The same trend can be observed in zinc and lead.

Although the removals are minor in the case of lead. Only 4% of iron can be removed from the quaternary solution. It is observed that in binary solutions, iron interferes more in Pb removal than in Cu and Zn removal. An interference trend can be established in the following binary solutions, Pb-Fe > Zn-Fe > Cu-Fe. In the quaternary solution a greater removal of zinc and copper is observed; and less lead removal is noticed. A removal trend can be established, Zn > Cu > Pb >> Fe.

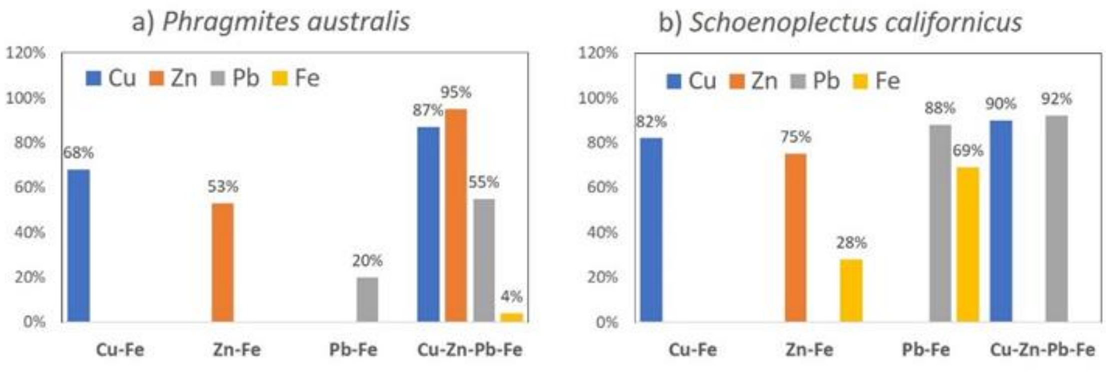

Figure 2: Metal removal percentages in each wetland

Table 4: Heavy metal concentrations [mg/l] in *Phragmites australis* determined by AAS.

Metal	Day	Solutions			
		Cu-Fe	**Zn-Fe**	**Pb-Fe**	**Cu-Zn-Pb-Fe**
Cu (mg/l)	Day (0)	0.50	---	---	0.97
	Day (9)	0.16	---	---	0.13
Zn (mg/l)	Day (0)	---	0.45	---	1.49
	Day (9)	---	0.21	---	0.08
Pb (mg/l)	Day (0)	---	---	0.46	0.31
	Day (9)	---	---	0.37	0.14
Fe (mg/l)	Day (0)	0.26	0.35	0.47	0.28
	Day (9)	0.64	1.33	0.54	0.27

- ***Schoenoplectus californicus***

The variation of the metal concentrations in binary and quaternary solutions are indicated in Fig. 2.b. Iron can only be removed in the quaternary solution and in the leaded binary solution. The removal of copper is greater in the quaternary solution than in the binary one. The same trend can be observed in lead and iron. Although the removals are minor in the case of iron. It is observed that in binary solutions, iron interferes more in Zn removal than in Cu and Pb removal. An interference trend can be established in the following binary solutions, Zn-Fe > Cu-Fe > Pb-Fe. In the quaternary solution a greater removal of lead and copper is observed. In this case, a trend can be established and removal, Pb > Cu.

Table 5: Heavy metal concentrations [mg/l] in *Schoenoplectus californicus* determined by AAS.

		Solutions			
Metal	**Day**	**Cu-Fe**	**Zn-Fe**	**Pb-Fe**	**Cu-Zn-Pb-Fe**
Cu (mg/l)	Day (0)	0.50	---	---	0.71
	Day (9)	0.09	---	---	0.07
Zn (mg/l)	Day (0)	---	0.83	---	0.24
	Day (9)	---	0.21	---	0.64
Pb (mg/l)	Day (0)	---	---	0.33	0.66
	Day (9)	---	---	0.04	0.05
Fe (mg/l)	Day (0)	0.26	0.18	0.29	0.36
	Day (9)	0.55	0.13	0.09	0.71

Metal accumulation in plant species

The final concentration of heavy metal in each part of the plants were measured with the method described in section 2.4. The results are summarized in Table 6 and Table 7. It is important to note that the Fe concentrations are the highest compared to the other heavy metals. Likewise, the Fe concentration in the in roots of *Schoenoplectus californicus* is higher than roots of the *Phragmites australis*.

- ***Phragmites australis***

In this case, the metals are adsorbed on the roots, with the highest root/stem ratio for Fe in the binary solution (Cu-Fe and Zn-Fe) and Cu in quaternary solution. A greater fixation of iron in the roots is observed for all solutions, being slightly greater for the binary solution of Zn-Fe.

In addition, it is observed that the *Phragmites australis* accumulates iron (95%) and copper (92%) in the root part in the binary (Cu-Fe) and quaternary solution respectively. Near 65% of Pb is accumulate in the roots in binary (Pb-Fe) and quaternary solution. The translocation factor (TF) shows that the plant is a phytostabilizer.

Table 6: **Final concentrations (ppm) of heavy metals in different parts of *Phragmites australis*.**

Metal	Part	Solutions			
		Cu-Fe	Zn-Fe	Pb-Fe	Cu-Zn-Pb-Fe
Cu (mg/l)	Roots	0.95	---	---	2.44
	Stems	0.55	---	---	0.22
	TF	0.58	---	---	0.09
Zn (mg/l)	Roots	---	1.47	---	2.36
	Stems	---	0.26	---	1.08
	TF	---	0.18	---	0.46
Pb (mg/l)	Roots	---	---	0.82	0.56
	Stems	---	---	0.47	0.29
	TF	---	---	0.57	0.51
Fe (mg/l)	Roots	27.35	36.30	22.25	28.10
	Stems	1.59	2.69	6.63	4.08
	TF	0.06	0.07	0.30	0.15

Table 7: **Final concentrations (ppm) of heavy metals in different parts of *Schoenoplectus californicus*.**

Metal	Part	Solutions			
		Cu-Fe	Zn-Fe	Pb-Fe	Cu-Zn-Pb-Fe
Cu (mg/l)	Roots	7.36	---	---	5.14
	Stems	0.80	---	---	0.39
	TF	0.11	---	---	0.08
Zn (mg/l)	Roots	---	2.19	---	4.38
	Stems	---	0.87	---	1.38
	TF	---	0.40	---	0.31
Pb (mg/l)	Roots	---	---	2.07	1.42
	Stems	---	---	1.09	1.59
	TF	---	---	0.53	1.12
Fe (mg/l)	Roots	102.30	110.93	160.23	107.91
	Stems	10.29	19.68	6.30	13.51
	TF	0.10	0.18	0.04	0.13

- ***Schoenoplectus californicus***

In this case, the metals are adsorbed on the roots. With the exception of lead in the quaternary solution where this metal is absorbed in the stem. The highest root/stem ratio is observed for Fe in the binary solution

(Pb-Fe) and Cu in the quaternary solution. Likewise, it is observed that the *Schoenoplectus californicus* mainly retains the ions in its root, copper (93%), zinc (76%) and iron (96%). The lowest root metal content is observed for Pb in the quaternary solution. The translocation factor (TF) shows that the plant is a phytostabilizer.

Conclusion

Phragmites australis (cav.) trin. ex steud. and *Schoenoplectus californicus (C.A. Mey.)* can be successfully remove heavy metals as Cu and Zn; and to a lesser extent Pb.

In wetlands with *Phragmites australis (cav.) trin. ex steud*., Fe appears to interfere with the removal of other metals when it is in a binary solution, with its greatest impact on lead removal (only 20%). However, in the presence of more metals, the interference of iron decreases considerably, allowing a good removal of Cu (87%) and Zn (95%); and greatly improving the removal of Pb (55%).

In wetlands with *Phragmites australis (cav.) trin. ex steud*., the interference of Fe seems to be less, especially in binary solutions, in which despite its presence, a good percentage of Cu (82%), Zn (75%) and Pb (88%) can still be removed. In the quaternary solution, a preferential recovery of Cu (90%) and Pb (92%) is observed, to the detriment of Zn and Fe.

In general, we can conclude that the studied plants have a potential to be used in wetlands. This allows having scientific support for the replication of this process with these plants, either in the laboratory under different conditions, or on a larger scale. Nonetheless, the effect of the species in Fe concentration requires additional study due to the reverse effect that has been observed.

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